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AQUATIC LIFE AMBIENT FRESHWATER QUALITY CRITERIA - COPPER

2007 Revision

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U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
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NOTICES

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http://www.epa.gov/waterscience/criteria/aqlife.html

FOREWORD

Section 304(a)(l) of the Clean Water Act of 1977 (P.L. 95-217) requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document is a revision of criteria based upon consideration of comments received from independent peer reviewers and the public. Criteria contained in this document supplement any previously published EPA aquatic life criteria for the same pollutant(s).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(l) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of health or ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific waterbody uses are adopted by a state or tribe as water quality standards under section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states or tribes might want to adjust water quality criteria developed under section 304 to reflect local environmental conditions. Alternatively, states or tribes may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as part of state or tribal water quality standards that criteria become regulatory. Guidelines to assist the states and tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 1994). The handbook and additional guidance on the development of water quality standards and other water-related programs of this agency have been developed by the Office of Water.

This document is guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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ACRONYMS

ACR	Acute-Chronic Ratio
BL	Biotic Ligand
BLM	Biotic Ligand Model
CCC	Criterion Continuous Concentration
CF	Conversion Factors
CMC	Criterion Maximum Concentration
CWA	Clean Water Act
DIC	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EC	Effect Concentration
EPA	Environmental Protection Agency
FACR	Final Acute-Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
FIAM	Free Ion Activity Model
GMAV	Genus Mean Acute Value
GSIM	Gill Surface Interaction Model
LC50	Lethal Concentration at 50 Percent Effect Level
LOAEC	Lowest Observed Adverse Effect Concentration
NASQAN	National Stream Quality Accounting Network
NOAEC	No Observed Adverse Effect Concentration
pH	Negative logarithm of the concentration (mol/L) of the $H_3O^+[H^+]$ ion; scale range from 0 to 14
SMAV	Species Mean Acute Values
STORET	EPA STOrage and RETrieval Data System
WER	Water-Effect Ratio
WET	Whole Effluent Toxicity
WQC	Water Quality Criteria

1.0 INTRODUCTION

Copper is an abundant trace element found in the earth's crust and is a naturally occurring element that is generally present in surface waters (Nriagu, 1979). Copper is a micronutrient for both plants and animals at low concentrations and is recognized as essential to virtually all plants and animals (Kapustka et al., 2004). However, it may become toxic to some forms of aquatic life at elevated concentrations. Thus, copper concentrations in natural environments, and its biological availability, are important. Naturally occurring concentrations of copper have been reported from 0.03 to 0.23 µg/L in surface seawaters and from 0.20 to 30 µg/L in freshwater systems (Bowen, 1985). Copper concentrations in locations receiving anthropogenic inputs can vary anywhere from levels that approach natural background to 100 µg/L or more (e.g., Lopez and Lee, 1977; Nriagu, 1979; Hem, 1989) and have in some cases been reported in the 200,000 µg/L range in mining areas (Davis and Ashenberg, 1989; Robins et al., 1997). Mining, leather and leather products, fabricated metal products, and electric equipment are a few of the industries with copper-bearing discharges that contribute to anthropogenic inputs of copper to surface waters (Patterson et al., 1998).

Over the past 20 years, the U.S. Environmental Protection Agency (EPA) has published a number of guidance documents containing aquatic life criteria recommendations for copper (e.g., U.S. EPA 1980, 1985, 1986, 1996). The present document contains EPA's latest criteria recommendations for protection of aquatic life in ambient freshwater from acute and chronic toxic effects from copper. These criteria are based on the latest available scientific information, supplementing EPA's previously published recommendations for copper. This criteria revision incorporated new data on the toxicity of copper and used the biotic ligand model (BLM), a metal bioavailability model, to update the freshwater criteria. With these scientific and technical revisions, the criteria will provide improved guidance on the concentrations of copper that will be protective of aquatic life. The BLM is not used in the saltwater criteria derivation because further development is required before it will be suitable for use to evaluate saltwater data.

This document provides updated guidance to states and authorized tribes to establish water quality standards under the Clean Water Act (CWA) to protect aquatic life from elevated copper exposure. Under the CWA, states and authorized tribes are to establish water quality criteria to protect designated uses. Although this document constitutes EPA's scientific recommendations regarding ambient concentrations of copper, it does not substitute for the CWA or EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, states, tribes, or the regulated community, and might not apply to a particular situation based on the circumstances. State and tribal decision makers retain the discretion in adopting approaches, on a case-by-case basis, that differ from this guidance when appropriate. EPA may change this guidance in the future.

Although the BLM has been used in place of the formerly applied hardness-based approach, the updated freshwater criteria derivations in this document are still based on the principles set forth in the *Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Life and Their Uses* (Stephan et al. 1985, hereafter referred to as the Guidelines). Section 2 of this document provides an overview of copper bioavailability and the BLM. Additional information on the generalized BLM framework, theoretical background, model calibration, and application for the BLM can be found in the published literature. Section 3 of this document discusses general

procedures and requirements for applying the BLM to criteria. Section 4 provides the derivation of criteria Final Acute Value (FAV) and Final Chronic Value (FCV) for freshwater organisms. Section 5 discusses plant data and Section 6 discusses other data not included in the criteria derivation. Sections 7 and 8 provide the final criteria statements and information on implementation. Various supplementary information is provided in several appendices.

2.0 APPROACHES FOR EVALUATING COPPER BIOAVAILABILITY

2.1 *General Aspects of Copper Bioavailability*

The toxicity of a chemical to an aquatic organism requires the transfer of the chemical from the external environment to biochemical receptors on or in the organism at which the toxic effects are elicited. Often, this transfer is not simply proportional to the total chemical concentration in the environment, but varies according to attributes of the organism, chemical, and exposure environment so that the chemical is more or less "bioavailable". Definitions of bioavailability vary markedly (e.g., National Research Council, 2003) and are often specific to certain situations, but a useful generic definition is the relative facility with which a chemical is transferred from the environment to a specified location in an organism of interest.

Of particular importance to bioavailability is that many chemicals exist in a variety of forms (chemical species). Such chemical speciation affects bioavailability because relative uptake rates can differ among chemical species and the relative concentrations of chemical species can differ among exposure conditions. At equilibrium in oxygenated waters, "free" copper exists as cupric ion - Cu(II) weakly associated with water molecules ($Cu{\cdot}nH_2O^{+2}$), but this species is usually a small percentage of the total copper. Most dissolved copper is part of stronger complexes with various ligands (complexing chemicals that interact with metals), including dissolved organic compounds, hydroxides, carbonates, and other inorganic ligands. Substantial amounts of copper can also be adsorbed to or incorporated into suspended particles. More information on copper speciation in freshwater can be found in Kramer et al. (1997), Bryan et al. (2002), and Smith et al. (2002).

Copper toxicity has been reported to vary markedly due to various physicochemical characteristics of the exposure water (e.g., either laboratory or field), including temperature, dissolved organic compounds, suspended particles, pH, and various inorganic cations and anions, including those composing hardness and alkalinity (see reviews by Sprague, 1968; Hunt, 1987; Campbell, 1995; Allen and Hansen, 1996; Paquin et al., 2002). Many of these physicochemical factors affect copper speciation, and their effects on copper toxicity therefore could be due to effects on copper bioavailability. That bioavailability is an important factor is evident from uptake of copper by aquatic organisms being reduced by various organic compounds and inorganic ligands known to complex copper (Muramoto, 1980; Buckley et al., 1984; Playle et al., 1993 a,b; MacRae et al., 1999).

A "ligand" is a complexing chemical (ion, molecule, or molecular group) that interacts with a metal like copper to form a larger complex. A "biotic ligand" is a complexing chemical that is a component of an organism (e.g. chemical site on a fish gill). For certain ligands, some studies have demonstrated that the concentration of free copper associated with a specified level of accumulation or toxicity changes little as the ligand concentration is varied, despite major changes in the

proportion of copper bound to the ligand (see review by Campbell, 1995). This suggests that, even at low concentrations, free copper is more important to bioavailability than the ligand-bound copper. This is expected if accumulation and toxicity are dependent on the binding of copper to a biochemical receptor "X" on the surface of the organism, forming a chemical species X-Cu (receptor-bound metal) that is a first limiting step in accumulation and toxicity. By standard chemical equilibrium expressions, the amount of such species and the consequent biological effects would be a function of the activity of just free copper (Morel, 1983 a), a relationship commonly referred to as the free ion activity model (FIAM). Ligand-bound copper (Cu-L) would contribute to copper bioavailability if (a) a species X-Cu-L is formed that is important to copper accumulation/toxicity, (b) the microenvironment near the organism surface is such that Cu-L dissociates and increases the free copper activity interacting with "X", or (c) copper uptake is via mechanisms that do not entail binding to such a receptor and can accommodate different copper species. Some studies have indicated dissolved complexes of copper do contribute to bioavailability (reviews by Sprague, 1968; Hunt, 1987; Campbell, 1995; Allen and Hansen, 1996; Paquin et al., 2002).

The effects of physicochemical factors on copper toxicity are diverse and the specific chemistry of the exposure water will determine whether or not there are appreciable effects on copper speciation and a resulting strong relationship of toxicity to free copper. Usually copper toxicity is reduced by increased water hardness (reviews by Sprague, 1968; Hunt, 1987; Campbell, 1995; Allen and Hansen, 1996; Paquin et al., 2002), which is composed of cations (primarily calcium and magnesium) that do not directly interact with copper in solution so as to reduce bioavailability. In some cases, the apparent effect of hardness on toxicity might be partly due to complexation of copper by higher concentrations of hydroxide and/or carbonate (increased pH and alkalinity) commonly associated with higher hardness. However, significant effects on toxicity often are still present when hardness is increased in association with anions which do not interact strongly with copper (Inglis and Davis, 1972; Chakoumakos et al., 1979; Miller and Mackay, 1980; Erickson et al., 1987). Hardness cations could have some limited effect on copper speciation by competing with copper for the same dissolved ligands, but increased hardness would then increase free copper and thus increase, not decrease, toxicity. Sodium has also been reported to affect copper toxicity (Erickson et al., 1996 b) and pH effects can be partly due to effects of hydrogen ion other than on copper speciation (Peterson et al., 1984).

The effects of hardness cations could be explained by the competing with copper for the biochemical receptor "X", thus reducing copper uptake (Zitko, 1976; Zitko et al., 1976; Pagenkopf, 1983). Reduced metal bioavailability due to increased hardness cations has been experimentally demonstrated (Playle et al., 1992; Meyer et al., 1999, 2002), although this does not specifically establish cation competition as the mechanism. Pagenkopf (1983) provided a mathematical description of a Gill Surface Interaction Model (GSIM) that addressed the effects on metal toxicity of both metal speciation and cations via the interactions of gill surface biochemical receptors with the free toxic metal, other metal species, hardness cations, and hydrogen ion.

The empirical evidence demonstrates that copper toxicity is affected by exposure conditions and that much of these effects is plausibly attributed to effects of ligands and cations on copper bioavailability. However, it should not be presumed that all of the observed effects of the physicochemical factors on copper toxicity reflect effects on bioavailability, or that bioavailability

effects are just due to ligand complexation and cation competition. For example, acute copper toxicity in aquatic organisms has been related to disruption of osmoregulation, specifically sodium/potassium exchange (Lauren and MacDonald, 1986; Wood, 1992; Wood et al., 1997; Paquin et al., 2002), which can be affected by calcium other than by competition with copper for the same biochemical receptor. Similarly, reported effects of sodium and potassium on copper toxicity (Erickson et al., 1996 b) might simply reflect favorable or unfavorable ion exchange gradients, rather than any effect on copper bioavailability. Nevertheless, the effects of ligand complexation and cation competition on copper bioavailability provide a reasonable conceptual framework for improved descriptions of how copper toxicity differs across exposure conditions.

2.2 *Existing Approaches*

EPA aquatic life criteria for metals address the reported effects of hardness on metal toxicity using empirical regressions of toxic concentrations versus hardness for available toxicity data across a wide range of hardness (Stephan et al., 1985). Such regressions provided the relative amount by which the criteria change with hardness, but have certain limitations. The regressions were not just of hardness, but of any other factor that was correlated with hardness in the toxicity data set used for the regressions, particularly pH and alkalinity. Although these regressions therefore address more bioavailability issues than hardness alone, they best apply to waters in which the correlations among hardness, pH, and alkalinity are similar to the data used in the regressions. The separate effects of these factors are not addressed for exposure conditions in which these correlations are different. In addition, some physicochemical factors affecting metal toxicity, such as organic carbon, are not addressed at all.

Existing EPA metals criteria also address bioavailability by using dissolved metal as a better approximation for metal bioavailability than total metal (U.S. EPA, 1993). Although this approach accounts for the low bioavailability of metal on suspended particles, it does not address the major effects of various dissolved species on bioavailability. This approach could conceivably be further developed to include just part of the dissolved copper, but this not only requires resolving what species to include, how to weight them, and how to assess their concentrations, but also would not address the effects of cations and other factors that affect toxicity in addition to metal speciation. Such a "bioavailable fraction" approach is not justified, because no fraction of metals species provides a constant measure of toxicity.

To address more completely the modifying effects of water quality than the hardness regressions achieve, EPA issued guidance in the early 1980s on the water-effect ratio (WER) method (Carlson et al., 1984; U.S. EPA, 1983, 1992, 1994). The WER is "a biological method to compare bioavailability and toxicity in receiving waters versus laboratory test waters" (U.S. EPA, 1992). A WER is calculated by dividing the acute LC50 of the metal, determined in water collected from the receiving water of interest, by the LC50 of the metal determined in a standard laboratory water, after adjusting both test waters to the same hardness. The standard laboratory water LC50 is used as the denominator to reflect that this LC50 is measured in test water that has water quality characteristics representative of the test waters used to develop the Water Quality Criteria (WQC) toxicity database, at least as a good approximation. The national hardness-based acute criterion concentration is then multiplied by this ratio (i.e., the WER) to establish a site-specific criterion that reflects the effect of site water characteristics on toxicity. However, a WER accounts only for

interactions of water quality parameters and their effects on metal toxicity to the species tested and in the water sample collected at a specific location and at a specific time. There is also significant cost to generate a single WER.

Because of the limitations of these past approaches for addressing bioavailability in metals criteria, there is a need for an approach that (1) explicitly and quantitatively accounts for the effect of individual water quality parameters that modify metal toxicity and (2) can be applied more cost-effectively and easily, and hence more frequently across spatial and temporal scales. An assessment framework that incorporates the bioavailability mechanisms discussed in Section 2.1 was therefore used to address more comprehensively the effects of physicochemical exposure conditions on copper toxicity with lower costs than required by the WER approach.

2.3 *The Biotic Ligand Model and Its Application to Criteria Development*

The interactions of toxic metal species and other exposure water constituents with biological surface receptors described by Zitko (1976), Morel (1983), and Pagenkopf (1983) provided the basic conceptual and mathematical structure for the bioavailability model to be used here (Figure 1). Subsequent experimental work has supported various model tenets by demonstrating the effects of complexing ligands and competing cations on accumulation of toxic metals at fish gills and the relationship of toxic effects to accumulation, and has also provided estimates of various model parameters (Playle et al., 1992, 1993a,b; Janes and Playle, 1995; MacRae et al., 1999, Meyer et al., 1999, 2002; McGeer et al., 2002). Various efforts in metal speciation modeling also have provided the ability to do better speciation calculations, especially regarding complexation of metals by organic matter (e.g., Tipping, 1994). This experimental work has supported further metal toxicity model development (Meyer, 1999; Brown and Markich, 2000; McGeer et al., 2002; Di Toro et al., 2001; Santore et al., 2001; Paquin et al., 2002). This bioavailability modeling approach is now commonly termed "Biotic Ligand Models" to broaden the scope beyond gill surfaces and to acknowledge that the biochemical receptor "X" discussed in Section 2.1 is a metal-binding ligand that is treated similarly to ligands in the exposure water, except that it is on the organism and is the keystone for metal accumulation and toxicity.

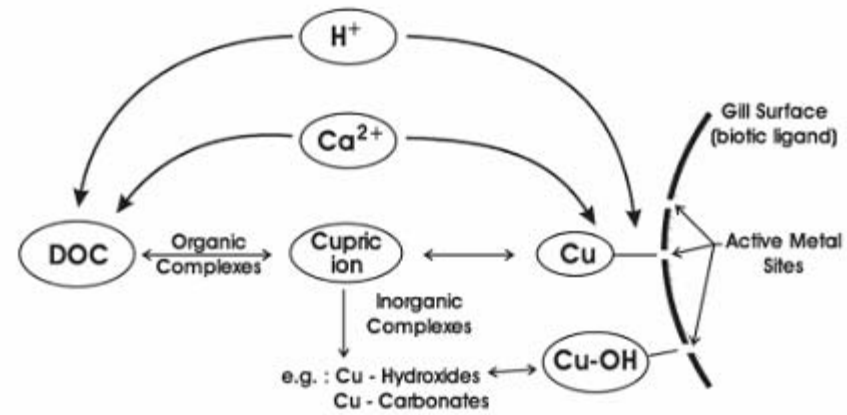

Figure1. Conceptual Diagram of Copper Speciation and Copper-Gill Model (after Pagenkopf, 1983)

Briefly, available evidence indicates that both free copper and copper monohydroxide bind to a biotic ligand "Lb" on the organism's surface (Lb-Cu and Lb-CuOH) and that death occurs when a certain amount of the total biotic ligand sites are occupied by copper. This ligand must be at the organism surface because the model describes its interactions with the external exposure water. However, this does not mean that this ligand is the site of toxic action; rather it is only necessary to assume that copper accumulation at the site(s) of toxic action is proportional to binding at the biotic ligand (i.e., the biotic ligand controls bioavailability). Other cations also will bind to the biotic ligand, affecting copper bioavailability because higher concentrations of copper are needed for copper to reach toxic levels. The binding to the biotic ligand is considered to be at equilibrium, with apparent (activity-corrected) equilibrium constants K_{LbCu}, K_{LbCuOH}, and K_{LbCj}, respectively, for free copper, copper hydroxide, and the "jth" competing cation. Chemical speciation in the exposure water is also considered to be at equilibrium, and chemical speciation calculations are conducted to compute the free copper, copper hydroxide, and competing cation activities to which the biotic ligand is exposed. Because binding to the actual biotic ligand cannot be measured, it is expected that accumulation relationships for some measurable variable (e.g., the total metal in gill tissue) provide a reasonable surrogate for the actual biotic ligand. Because criteria deal with concentrations eliciting a certain level of effects on groups of organisms (e.g., LC50s), model calculations are for an organism with characteristics appropriate for such group-wide statistics.

How the BLM is applied to criteria can be best discussed by starting with the following general expression for the BLM:

$$EC = EC_0 \cdot f_C \cdot f_L \qquad \text{Equation 1}$$

where EC is the total dissolved copper concentration eliciting an effect, EC_0 is a baseline EC in the absence of any complexing ligands and competing cations, f_C should be a factor (<1) for how much competing cations increase EC, and f_L should be a factor (<1) for how much complexing ligands increase EC. For the BLM used here:

$$EC_0 = \frac{f_{LbT}}{(1 - f_{LbT}) \cdot K_{LbCu}} \qquad \text{Equation 2}$$

$$f_C = 1 + \sum_j^m \left(K_{Cj,Lb} \cdot [C_j]\right) \qquad \text{Equation 3}$$

$$f_L = \frac{1}{\alpha_{Cu^{+n}} + \frac{K_{LbCuOH}}{K_{LbCu}} \cdot \alpha_{CuOH}} \qquad \text{Equation 4}$$

where f_{LbT} is the fraction of the biotic ligand sites that must be occupied by copper to elicit the toxicity of interest (e.g., a lethal accumulation divided by the accumulation capacity), *m* is the

number of competing cations included in the model, [Cj] is the concentration of the jth competing cation, α_{Cu+2} is the ratio of free copper concentration to total dissolved copper concentration, α_{CuOH} is the ratio for the copper hydroxide complex, and the ratio K_{LbCuOH}/K_{LbCu} specifies the bioavailability of CuOH relative to free copper. Thus, in the absence of complexing ligands and competing cations, the toxic concentration is only a function of the binding strength of free copper and the copper occupied fraction of biotic ligand sites needed to elicit toxicity. The increase in the effect concentration due to competing cations is simply a sum of the products of their concentrations and binding constants. The increase in the effect concentration due to complexing ligands is the inverse of the sum of the products of the relative bioavailabilities and concentration fractions of the species that bind to the biotic ligand (free copper and copper hydroxide).

If toxicity to all the biological species in the criteria (at least the most sensitive ones) were determined based on measured accumulation properties and the relationship of toxicity to accumulation, the above model equations would be directly applied in criteria calculations. However, this is not the case. Although gill accumulation properties and lethal accumulations have been measured for certain species and conditions, and this has been useful in validating BLM assumptions and formulations, the data that must be applied to the criteria consists of water effect concentration (ECs) for biological species for which this accumulation information is generally not available. The BLM therefore is needed, not to make absolute calculations regarding toxic concentrations, but to extrapolate toxic concentrations from one exposure condition to another:

$$EC_A = EC_B \cdot \frac{f_{C,A} \cdot f_{I,A}}{f_{C,B} \cdot f_{I,B}} \qquad \text{Equation 5}$$

where the A and B subscripts refer to different exposure conditions. The general procedure that was followed for criteria development here was to use the above equation to normalize all available toxicity data to a reference exposure condition, calculate criteria values at the reference condition, and again use the above equation to compute criteria at other conditions.

This means that the BLM assumptions and parameters that just pertain to EC_0 are not important to its application to criteria, which actually simplifies model validation and parameterization needs. In particular, there is no need to estimate f_{LbT}, or the lethal accumulations and accumulation capacities that define this fraction. Furthermore, the absolute values of K_{LbCu} and K_{LbCuOH} do not need to be known, only their relative value (and if copper binding to the biotic ligand was dependent only on free copper, the value of K_{LbCu} would not be needed at all). Absolute values are only needed for the binding constants for the competing cations, as well as the various constants needed in speciation calculations to estimate α_{Cu+2} and α_{CuOH}. For BLM application to criteria, the important concern is whether f_C and f_L are suitably formulated and parameterized, and not with issues that relate to lethal accumulations and accumulation capacities.

2.4 *BLM Uncertainties and Performance*

The BLM employed here uses equilibrium reactions of copper and other cations with a single, simple type of surface ligand as the focus for all the effects of physicochemical exposure conditions on toxicity, and thus is a simple, approximate representation for the complex set of chemical

reactions and transfers involved with environmental copper concentrations eliciting toxicity. As already noted, cation effects might involve mechanisms other than competition for a surface ligand. The microenvironment at the gill might change copper speciation. Multiple mechanisms that do not react the same to external conditions might be involved in copper bioavailability and toxicity. Accumulation parameters based on bulk gill measurements will likely not be the same as those for the biotic ligand. Nonequilibrium processes might be important, especially regarding the relationship of copper-binding on a surface ligand to toxic action.

However, any model is a simplification of reality and the existence of uncertainties does not preclude a model from being useful and justified. Despite its simplicity, the BLM used here provides a reasonable mechanistic framework for the well-established effects of copper speciation, explicitly addressing the relative bioavailability of different copper species. It also includes a plausible mechanism that allows the effects of cations to be addressed and uses a comprehensive model for calculating the required concentrations of various chemical species. Even if the mechanistic descriptions are incomplete, this model allows the major empirical effects of complexing ligands and competing cations to be described in a more comprehensive and reasonable fashion than other approaches.

Because this model is used in criteria to predict relative effects of physicochemical exposure factors, its utility for criteria can be judged based on how well it predicts the relative effects of these factors in copper toxicity studies. Examples of BLM performance for various exposure factors and studies are provided in the technical support document for this criteria. Figure 2 shows one example from a study on the effects of various exposure conditions on the acute lethality of copper to fathead minnows. This set of exposures consisted of synthetic exposure solutions of various total ion concentrations with fixed ratios of the major cations and anions, at a fixed pH (8.0) and low dissolved organic matter (< 0.5 mg/L). Observed dissolved LC50s (solid circles with uncertainty bars) varied by 24-fold for only a 9-fold change in total ions. These large effects reflect the combined influences of increased alkalinity (copper carbonate complex formation), hardness, and sodium. Considering the wide range of the observed LC50s and that the model was not fitted to these data, BLM-predicted LC50s (open symbols) were rather accurate, ranging from 55 to 87% (average 75%) of the observed value. More importantly for criteria, the predicted relative change across the range of total ion concentration was 20-fold, very close to that observed.

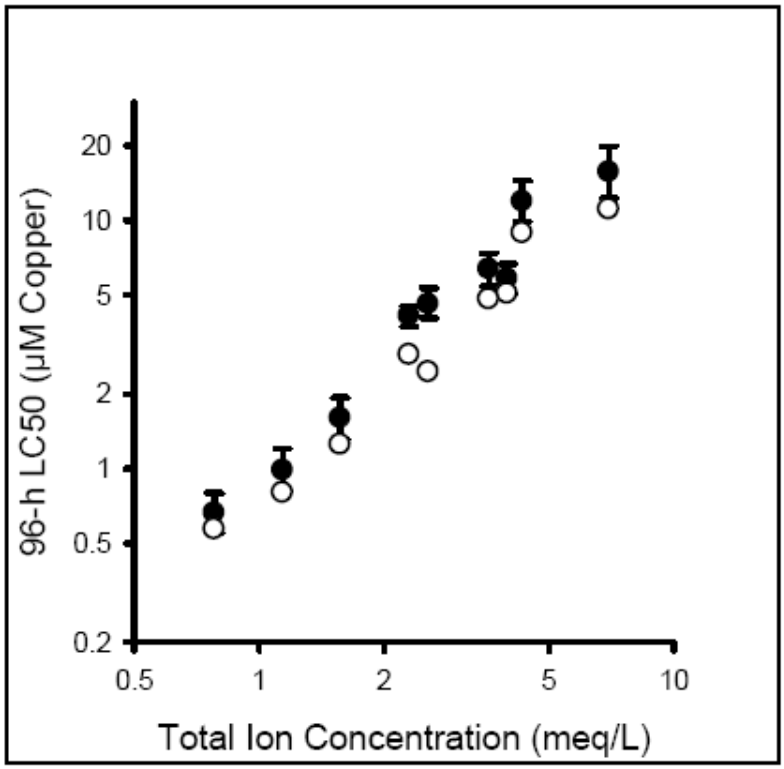

Figure 2. Effects of increasing total ion concentration on the acute lethality of copper to fathead minnows at constant pH=8 and low DOC < 0.5 mg/L. Solid symbols represent observed values, open symbols represent predicted values.

Model performance can also be judged across a variety of factors as in Figure 3, which shows predicted versus observed LC50s for a large number of exposures in the cited study, which varied hardness, alkalinity, sodium, and pH together and separately over a wide range. Observed LC50s varied by about 60-fold, but predicted values deviated from observed values by only 0.12 log units (a factor of 1.3) on average, and at worst only slightly more than a factor of 2. Again, more information on model performance is provided in the Technical Support Document and the figures here just provide some examples demonstrating the utility of this model for use in criteria.

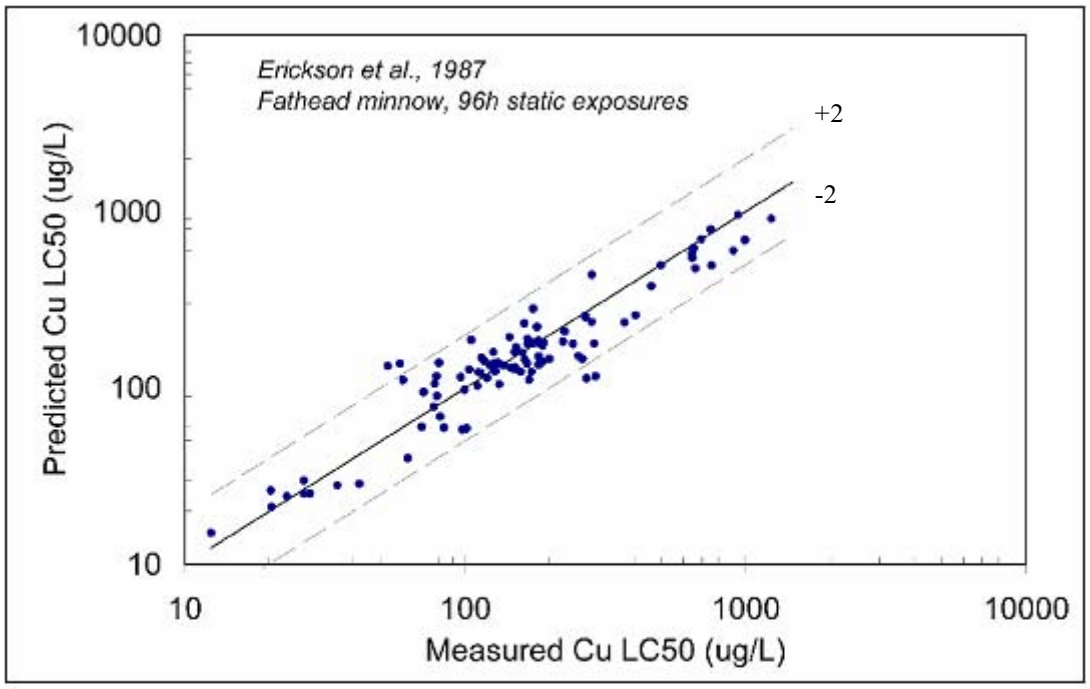

Figure 3. Comparison of Predicted and Measured Acute Copper Toxicity to *P. promelas*.

The use of the BLM to predict the bioavailability and toxicity of copper to aquatic organisms under site-specific conditions is a significant change from the previous Criterion Maximum Concentration (CMC) derivation methodology. Previous aquatic life criteria documents for copper (e.g., U.S. EPA, 1980, 1985, 1996) expressed the CMC as a function of water hardness. Now, EPA chooses to utilize the BLM to update its freshwater acute criterion because the BLM accounts for all important inorganic and organic ligand interactions of copper while also considering competitive interactions that influence binding of copper at the site of toxicity, or the "biotic ligand." The BLM's ability to incorporate metal speciation reactions and organism interactions allows prediction of metal effect levels to a variety of organisms over a wide range of water quality conditions. Accordingly, the BLM is an attractive tool for deriving water quality criteria. Application of the BLM has the potential to substantially reduce the need for site-specific modifications, such as Water Effect Ratio, to account for site-specific chemistry influences on metal toxicity.

The updated BLM-based WQC will in some cases be more stringent and in other cases less stringent than the hardness based WQC. As there is not a single WQC value to use for comparison purposes, it will only be possible to provide illustrative examples of each situation. It is the judgement of the EPA that the BLM-based WQC for Cu will provide an improved framework for evaluating a level of protection (LOP) that is consistent with the LOP that was intended by the 1985 Guidelines (i.e., a 1-in-3 year exceedance frequency that will be protective of 95% of the genera).

While the BLM is currently considered appropriate for use to derive an updated freshwater CMC for the acute WQC, further development is required before it will be suitable for use to

evaluate a saltwater CMC or a Criterion Continuous Concentration (CCC) or chronic value (freshwater or saltwater WQC).

3.0 INCORPORATION OF THE BLM INTO CRITERIA DERIVATIONS PROCEDURES

3.1 *General Final Acute Value (FAV) Procedures*

Application of the acute copper BLM to the derivation of the copper FAV is analogous to procedures already described in the Guidelines for metals criteria using empirical hardness regressions. For these hardness-dependent metals criteria, LC50s at various hardness are normalized to a reference hardness using the regression slopes. The normalized LC50s for each biological species are averaged to derive Species Mean Acute Values (SMAVs) at the reference hardness. The SMAVs within each genus are then averaged to derive Genus Mean Acute Values (GMAVs) at the reference hardness. The Guidelines' procedures for estimating the fifth percentile of the GMAVs are then used to derive the FAV at the reference hardness. FAVs for other hardness can then be derived using the hardness regression slope, and these FAVs are used to calculate the Criterion Maximum Concentration (CMC) by dividing the FAV by 2.0 and the Final Chronic Values (FCV) by dividing the FAV by the Final Acute-Chronic Ratio (FACR). Following the Guidelines, the Criterion Continuous Concentration (CCC) is set to the FCV unless other data justifies a lower value.

Extending this procedure to apply the BLM simply involves normalizing the LC50s to a reference exposure condition that includes all the physicochemical exposure factors important to the BLM, not just hardness. For this normalization, the BLM provides the factors f_C and f_L discussed in Section 2.3, these factors serving the same purpose as the hardness regression slope described above. Each LC50 to be used in criteria derivation would be normalized to the reference exposure conditions by the equation:

$$LC50_R = LC50_A \cdot \frac{f_{C,R} \cdot f_{L,R}}{f_{C,A} \cdot f_{L,A}} \qquad \text{Equation 6}$$

where the subscript A refers to the exposure conditions for the observed LC50 and the subscript R refers to the reference exposure conditions to which the LC50 is being normalized. These normalized LC50s are then used to derive the SMAVs, GMAVs, and FAV at the reference exposure condition as described above for the hardness-corrected criteria. The BLM is then used to derive FAVs at other exposures by the equation:

$$FAV_B = FAV_R \cdot \frac{f_{C,B} \cdot f_{L,B}}{f_{C,R} \cdot f_{L,R}} \qquad \text{Equation 7}$$

where the subscript B refers to the exposure conditions for which an FAV is desired. These BLM-derived FAVs are then used to derive CMCs and CCCs following standard Guidelines procedures.

For the criteria in this document, the reference exposure conditions to which LC50s are normalized and at which the reference FAV is calculated are as follows (see also footnote f in Table 1). The water chemistry used in the normalization was based on the EPA formulation for moderately-hard reconstituted water, but any other water chemistry could have been used. In this formulation the parameters included: temperature = 20°C, pH = 7.5, DOC = 0.5 mg/L, Ca = 14.0 mg/L, Mg = 12.1 mg/L, Na = 26.3 mg/L, K = 2.1 mg/L, SO_4 =81.4 mg/L, Cl = 1.90 mg/L, Alkalinity = 65.0 mg/L and S = 0.0003 mg/L.

3.2 *BLM Input Parameters*

For applying an LC50 to criteria derivations and for determining an FAV at exposure conditions of interest, the necessary water quality input parameters for BLM calculations are temperature, pH, dissolved organic carbon, major geochemical cations (calcium, magnesium, sodium, and potassium), dissolved inorganic carbon (DIC, the sum of dissolved carbon dioxide, carbonic acid, bicarbonate, and carbonate), and other major geochemical anions (chloride, sulfate). DIC measurements are typically not made in the environment, and an alternative input parameter is alkalinity, which can be used with pH and temperature to estimate DIC. There is some evidence that other metals such as iron and aluminum can have an effect on copper toxicity to aquatic organisms, which might be due to interactions of these metals with the biotic ligand, effects of these metals on organic carbon complexation of copper, or adsorption of copper to iron and aluminum colloids which are present in filtrates used to measure dissolved copper. These metals are not currently included in routine BLM inputs, but users are encouraged to measure dissolved iron and aluminum as part of monitoring efforts to support possible future criteria applications.

A number of fixed parameters are also used in the BLM but are not required user inputs in criteria derivations. These include the variety of equilibrium constants used in copper speciation calculations, and also the binding constants for copper and various cations to the biotic ligand. The values for these constants were obtained from work by Playle and coworkers (Playle et al., 1992, 1993a,b) and also by inference from the relationship of toxicity to various water quality characteristics. More information about these parameters can be obtained from the technical support document.

3.3 *Data Screening Procedures*

To use a toxicity test in the derivation of BLM-based criteria, information must be available for the various water quality parameters described in Section 3.2. This is in contrast to past metals criteria, for which the only necessary water quality parameter was hardness. Many of these parameters are not routinely measured in toxicity tests and, if measured, are not necessarily reported in the primary literature for the test, especially for older toxicity tests. However, this information might be available from supplemental sources or be estimated based on other information. Therefore, in addition to reviewing the primary sources for relevant information,

additional efforts were made to obtain or estimate the necessary water quality parameters for as many of the available LC50s as possible.

A detailed description of these efforts is provided in Appendix C, Estimation of Water Chemistry Parameters for Acute Copper Toxicity Tests, and are summarized as follows. Reports of acute copper toxicity tests identified in literature searches were reviewed to identify LC50s for possible inclusion in the criteria derivation. In addition to test acceptability standards specified in the Guidelines, the current effort also required that the LC50s be based on measured copper concentrations. LC50s based on nominal concentrations have been used in previous criteria, but there are enough measured LC50s for copper that this was considered to be no longer warranted, especially considering the more advanced bioavailability assessments represented by the BLM. For the identified LC50s, the primary reports were reviewed to record all reported information on dilution and test water chemistry. Any additional references specified by the authors were also obtained and reviewed. If test waters were synthetically prepared based on specified formulas, these were used to estimate parameters as appropriate. When critical water chemistry parameters were not available, authors were contacted regarding unpublished information or to measure missing water chemistry parameters in dilution source waters. If primary or corresponding authors could not be contacted, an attempt was made to contact secondary authors or personnel from the laboratories where the studies were conducted. Where actual water chemistry data were unavailable, data from other studies with the same water source were used as surrogate values if appropriate. Absent this, the U.S. Geological Survey's National Stream Quality Accounting Network (NASQAN) and the EPA STOrage and RETrieval (STORET) were used to obtain data for ambient surface waters which were the source of water for a test. In some instances other available sources were contacted to obtained water chemistry data (e.g., city drinking water treatment personnel). The acquired data were scrutinized for representativeness and usefulness for estimating surrogate values to complete the water quality information for the dilution and/or test water that was used in the original studies. When the above sources could not be used, geochemical ion inputs were based on reported hardness measurements and regressions relationships constructed for the relationship of various ions to hardness from NASQAN data.

As with any modeling effort, the reliability of model output depends on the reliability of model inputs. Although the input data have been closely scrutinized, the reliability of the BLM-normalized LC50s are subject to the uncertainties of the estimation procedures described above. Therefore, a ranking system was devised to rank the quality of the chemical characterization of the test water. Studies with a rank of 1 contain all of the necessary parameters for BLM input based on measurements from either the test chambers or the water source. In general, studies in which the BLM input parameters were reported for test chamber samples take precedence over studies in which the parameters were reported only for the source water. A characterization ranking of 2 denotes those studies where not all parameters were measured, but reliable estimates of the requisite concentrations could be made. Similarly, a rank of 3 denotes studies in which all parameters except DOC were measured, but reliable estimates of DOC could be made. For the majority of the tests, a chemical characterization of 4+ was assigned because hardness, alkalinity, and pH were measured, and the ionic composition could be reliably estimated or calculated. A 4- was assigned to those studies conducted using standard reconstituted water in which hardness, alkalinity, or pH was either measured or referenced, and the recipe for the water is known (ASTM, 2000; U.S. EPA, 1993). The chemical characterization rank of 5 was ascribed to studies in which

one of the key parameters (DOC, Ca, pH, alkalinity) was not measured, and when it could not be reliably estimated. If two or more key parameters (DOC, Ca, pH, alkalinity) were not measured and could not be reliably estimated, a study was given a chemical characterization rank of 6. Studies receiving a quality rating of greater than 4+ (i.e., higher than 4) were not used in the criteria development procedures because the estimates for some of the key input parameters were not thought to be reliable, all other studies were used.

3.4 *Conversion Factors*

The LC50s used in deriving previous EPA metals criteria were based on total metal concentration (measured or nominal) and the criteria were consequently for total metals concentration. EPA afterwards made the decision that metals criteria should be based on dissolved metal because it was thought to better represent the bioavailable fraction of the metal (U.S. EPA, 1993). It was thus necessary to convert the criteria to a dissolved concentration basis. However, at that time, most toxicity tests reported only total concentration, so that a procedure was necessary to estimate the likely fractions of metals that were dissolved in typical toxicity tests. Studies were therefore conducted to determine these fractions under a variety of test conditions that mimicked the conditions in the tests used to derive the metals criteria (University of Wisconsin-Superior, 1995). These tests demonstrated high fractions of dissolved copper and resulted in a conversion factor (CF) of 0.96 for converting both the CMC and CCC for copper from a total to dissolved basis (Stephan, 1995). The BLM-derived criteria developed here also uses dissolved copper as the basis for criteria, assuming a negligible bioavailability for particulate copper. The conversion factor of 0.96 was also used to convert total to dissolved copper for any toxicity test for which dissolved copper measurements were not available.

3.5 *Final Chronic Value (FCV) Procedures*

Because the minimum eight family data requirements for chronic toxicity data were not met in order to calculate the FCV by the fifth percentile method used for the FAV and because insufficient information was available to develop a chronic BLM, EPA derived the CCC utilizing the Acute to Chronic Ratio (ACR) approach from the Guidelines (Stephan et al., 1985). To calculate the FCV at a specific water chemistry, the FAV at that chemistry is divided by the FACR. This entails the assumption that the acute BLM reasonably approximates the bioavailability relationships for chronic toxicity. Limited data available regarding effects of water chemistry on sublethal effects and chronic lethality do show substantial effects of organic matter, alkalinity, pH, and sodium (Winner, 1985; Erickson et al., 1996 a,b) similar to those in the acute BLM used here. For hardness, apparent effects are limited and uncertain, but the use of the acute BLM does not introduce major uncertainties in this regard because the effects of hardness by itself in the acute BLM are also limited.

4.0 DATA SUMMARY AND CRITERIA CALCULATION

4.1 *Summary of Acute Toxicity to Freshwater Animals and Criteria Calculation*

The screening procedure outlined in Sec. 3.3 (high quality data = 1, low quality data > 4, e.g. 4+) identified approximately 600 acute freshwater toxicity tests with aquatic organisms and copper

potentially acceptable for deriving criteria. Of these tests, approximately 100 were eliminated from the criteria derivation process because they did not report measured copper concentrations. Nearly 150 additional tests were eliminated from the calculation of the FAV because they received a quality rating of greater than 4 in the quality rating scheme described in section 3.3 described above.

Data from approximately 350 tests were used to derive normalized LC50 values, including 15 species of invertebrates, 22 species of fish, and 1 amphibian species (Table 1), representing 27 different genera. Species Mean Acute Values (SMAVs) at the reference chemistry were calculated from the normalized LC50s and Genus Mean Acute Values (GMAVs) at the normalization chemistry were calculated from the SMAVs.

SMAVs ranged from 2.37 µg/L for the most sensitive species, *Daphnia pulicaria*, to 107,860 µg/L for the least sensitive species, *Notemigonus crysoleucas*. Cladocerans were among the most sensitive species, with *D. pulicaria, D. magna*, *Ceriodaphnia dubia*, and *Scapholeberis sp.* being four out of the six most sensitive species. Invertebrates in general were more sensitive than fish, representing the 10 lowest SMAVs.

The 27 GMAVs calculated from the above-mentioned SMAVs ranged from 4.05 µg/L for *Daphnia* to 107,860 µg/L for *Notemigonus* (Table 3a). Nine of the 10 most sensitive genera were invertebrates. The salmonid genus *Oncorhynchus* was the most sensitive fish genus, with a GMAV of 31.39 µg/L and an overall GMAV ranking of 10.

The ranked GMAVs are presented in Figure 4. Pursuant to procedures used to calculate the FAV, a FAV of 4.67 µg/L was derived from the four GMAVs with cumulative probabilities closest to the 5th percentile toxicity value for all the tested genera (Table 3b). The presumption is that this

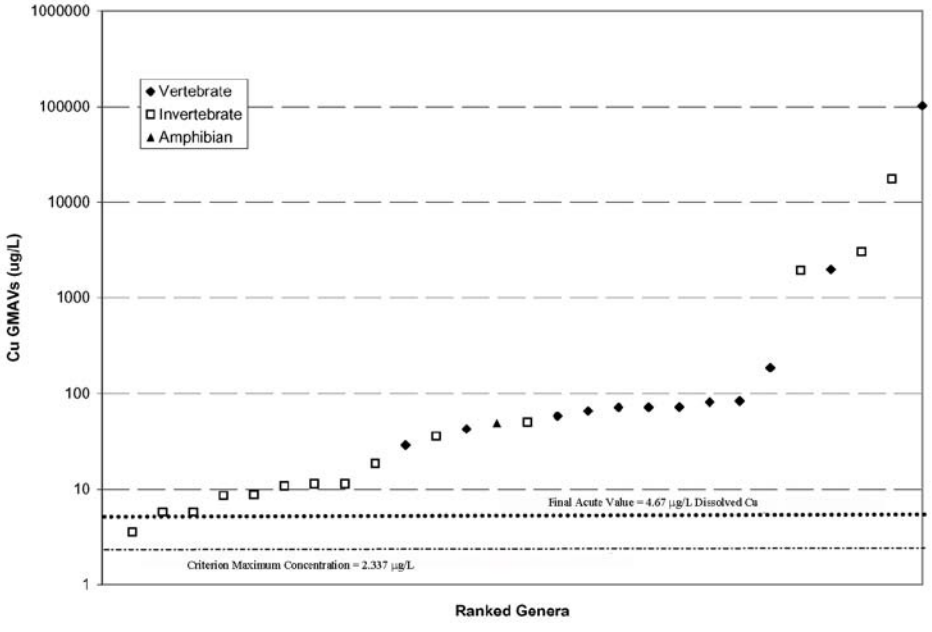

Figure 4. Ranked Freshwater Genus Mean Acute Values (GMAVs)

acute toxicity value represents the LC50 for an organism that is sensitive at the 5th percentile of the GMAV distribution. The CMC is the FAV divided by two. Therefore, the freshwater dissolved copper CMC for the reference chemistry presented is 2.337 µg/L.

Site-water chemistry parameters are needed to evaluate a criterion. This is analogous to the situation that previously existed for the hardness-based WQC, where a hardness concentration was necessary in order to derive a criterion. Examples of CMC calculations at various water chemistry conditions are presented in Figure 5 and Appendix G.

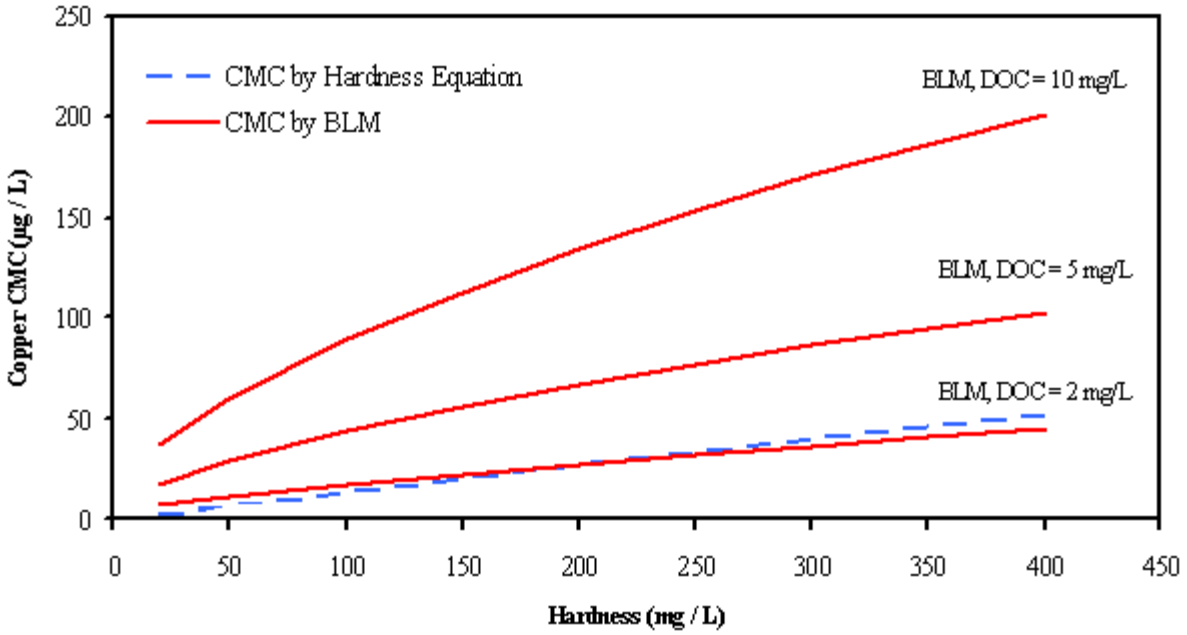

Figure 5. Comparison of CMC calculated by BLM or Hardness Equation Alkalinity (11 - 245 mg CaCO3/L) and pH (7.3 - 8.7) Covary with Hardness

4.1.1 *Comparison With Earlier Hardness-Adjusted Criteria*

EPA's earlier freshwater copper criteria recommendations were hardness-dependent values. One would expect a BLM-based criterion calculation procedure to yield the more appropriate criterion—appropriate in the sense that it accounts for the important water chemistry factors that affect toxicity, including DOC complexation, where the hardness correction does not. Application of the BLM in field situations where DOC is expected to be present at higher concentrations than those observed in laboratory studies would likely improve the performance of the BLM compared with the hardness adjustment. The reason is that the BLM would reasonably account for the typically observed increase in effect levels under such conditions, while the hardness-based approach would not (Figure 5).

As a comparison between the hardness typical of the previous copper criterion and this revised criterion using the BLM, both procedures were used to calculate criterion values for waters with a range in hardness as specified by the standard EPA recipes (U.S. EPA, 1993). The EPA formulations specify the concentration of various salts and reagents to be used in the synthesis of

laboratory test waters with specific hardness values (e.g., very soft, soft, moderately hard, hard, or very hard). As the water hardness increases in these recipes, pH and alkalinity also increase. This has implications for the BLM because the bioavailability of copper would be expected to decrease with increasing pH and alkalinity due to the increasing degree of complexation of copper with hydroxides and carbonates and decreasing proton competition with the metal at both DOM and biotic ligand binding sites. The BLM criterion for these waters agrees very well with that calculated by the hardness equation used in previous copper criterion documents (Figure 5). However, alkalinity and pH change as hardness changes in the EPA recipes. The BLM prediction is taking all of these changes in water quality into account.

It is possible to use the BLM to look only at the change in predicted WQC with changes in hardness (e.g., alkalinity and pH remaining constant). The hardness equation is based on waters where changes in hardness are accompanied by changes in pH and alkalinity. However, there are many possible natural waters where changes in hardness are not accompanied by changes in pH and alkalinity (such as water draining a region rich in gypsum). In these cases, the hardness equation based criterion will still assume a response that is characteristic of waters where hardness, alkalinity, and pH co-vary, and will likely be underprotective relative to the level of protection intended by the Guidelines, in high hardness waters. Conversely, in waters where the covariation between hardness, pH, and alkalinity is greater than is typical for data in Table 1, the hardness equation based criteria may be overprotective. Appendix G shows representative water quality criteria values using both the BLM and the hardness equation approaches for waters with a range in pH, hardness, and DOC concentrations. The hardness approach does not consider pH and DOC while the BLM approach takes those water quality parameters into consideration.

4.2 Formulation of the CCC

4.2.1 *Evaluation of Chronic Toxicity Data*

In aquatic toxicity tests, chronic values are usually defined as the geometric mean of the highest concentration of a toxic substance at which no adverse effect is observed (highest no observed adverse effect concentration, or NOAEC) and the lowest concentration of the toxic substance that causes an adverse effect (lowest observed adverse effect concentration, or LOAEC). The significance of the observed effects is determined by statistical tests comparing responses of organisms exposed to low-level and control concentrations of the toxic substance against responses of organisms exposed to elevated concentrations. Analysis of variance is the most common test employed for such comparisons. This approach, however, has the disadvantage of resulting in marked differences between the magnitudes of the effects corresponding to the individual chronic values, because of variation in the power of the statistical tests used, the concentrations tested, and the size and variability of the samples used (Stephan and Rogers, 1985).

An alternative approach to calculating chronic values focuses on the use of point estimates such as from regression analysis to define the dose-response relationship. With a regression equation or probit analysis, which defines the level of adverse effects as a function of increasing concentrations of the toxic substance, it is possible to determine the concentration that causes a specific small effect, such as a 5 to 30 percent reduction in response. To make chronic values reflect a uniform level of effect, regression and probit analyses were used, where possible, both to demonstrate that a significant concentration-effect relationship was present and to estimate chronic

values with a consistent level of effect. The most precise estimates of effect concentrations can generally be made for 50 percent reduction (EC50); however, such a major reduction is not necessarily consistent with criteria providing adequate protection. In contrast, a concentration that causes a low level of reduction, such as an EC5 or EC10, might not be statistically significantly different from the control treatment. As a compromise, the EC20 is used here to represent a low level of effect that is generally significantly different from the control treatment across the useful chronic datasets that are available for copper. The EC20 was also viewed as providing a level of protection similar to the geometric mean of the NOEC and LOEC. Since the EC20 is not directly dependent on the tested dilution series, similar EC20s should be expected irrespective of the tested concentrations, provided that the range of tested concentrations is appropriate.

Regression or probit analysis was utilized to evaluate a chronic dataset only in cases where the necessary data were available and the dataset met the following conditions: (1) it contained a control treatment (or low exposure data point) to anchor the curve at the low end, (2) it contained at least three concentrations, and (3) two of the data points had effect variable values below the control and above zero (i.e., "partial effects"). Control concentrations of copper were estimated in cases where no measurements were reported. These analyses were performed using the Toxicity Relationship Analysis Program software (version 1.0; U.S. EPA, Mid-Continental Ecology Division, Duluth, MN, USA). Additional detail regarding the aforementioned statistical procedures is available in the cited program.

When the data from an acceptable chronic test met the conditions for the logistic regression or probit analysis, the EC20 was the preferred chronic value. When data did not meet the conditions the chronic value was usually set to the geometric mean of the NOAEC and the LOAEC. However, when no treatment concentration was an NOAEC, the chronic value is reported as less than the lowest tested concentration.

For life-cycle, partial life-cycle, and early life stage tests, the toxicological variable used in chronic value analyses was survival, reproduction, growth, emergence, or intrinsic growth rate. If copper apparently reduced both survival and growth (weight or length), the product of variables (biomass) was analyzed, rather than analyzing the variables separately. The most sensitive of the toxicological variables was generally selected as the chronic value for the particular study.

A species-by-species discussion of each acceptable chronic test on copper evaluated for this document is presented in Appendix F. Figures that present the data and regression/probability distribution line for each of the acceptable chronic test which contained sufficient acceptable data are also provided in Appendix F.

4.2.2 *Calculation of Freshwater CCC*

Acceptable freshwater chronic toxicity data from early life stage tests, partial life-cycle tests, and full life-cycle tests were available for 29 tests including data for 6 invertebrate species and 10 fish species (Table 2a). The 17 chronic values for invertebrate species range from 2.83 (*D. pulex)* to 34.6 µg/L (*C. dubia*); and the 12 chronic values for the fish species range from <5 (brook trout) to 60.4 µg/L (northern pike). Of the 29 chronic tests, comparable acute values are available for 18 of the tests (Table 2c). The relationship between acute toxicity values and ACRs is presented in Figure 6. The supporting acute and chronic test values for the ACRs and the species mean ACRs are

presented in Table 2c. For the 11 tests in Table 2a with chronic values both from a regression EC20 and the geometric mean of the NOAEC and LOAEC, the EC20 averaged 81% of the geometric mean, demonstrating the similar level of protection for the two approaches.

Overall, individual ACRs varied from <1 (0.55) for *C. dubia* (Oris et al., 1991) to 191.6 for the snail, *Campeloma decisum* (Arthur and Leonard, 1970). Species mean acute-chronic ratios ranged from 1.48 in saltwater for the sheepshead minnow (Hughes et al., 1989) to 171.2 in freshwater for the snail, *C. decisum*. Pursuant to the Guidelines (Stephan et al., 1985), consideration was given to calculating the FACR based on all ACRs within a factor of 10, but because there appeared to be a relationship between acute sensitivity and ACRs (Figure 6), the FACR was derived from data for species whose SMAVs were close to the FAV. The FACR of 3.22 was calculated as the geometric mean of the ACRs for sensitive freshwater species, *C. dubia, D. magna, D. pulex, O. tshawytscha*, and *O. mykiss* along with the one saltwater ACR for *C. variegatus* (Table 2b). Based on the normalization water chemistry conditions used for illustrative purposes in the document, the freshwater site specific FAV value is 4.67 µg/L, which divided by the FACR of 3.22 results in a freshwater FCV of 1.45 µg/L dissolved Cu.

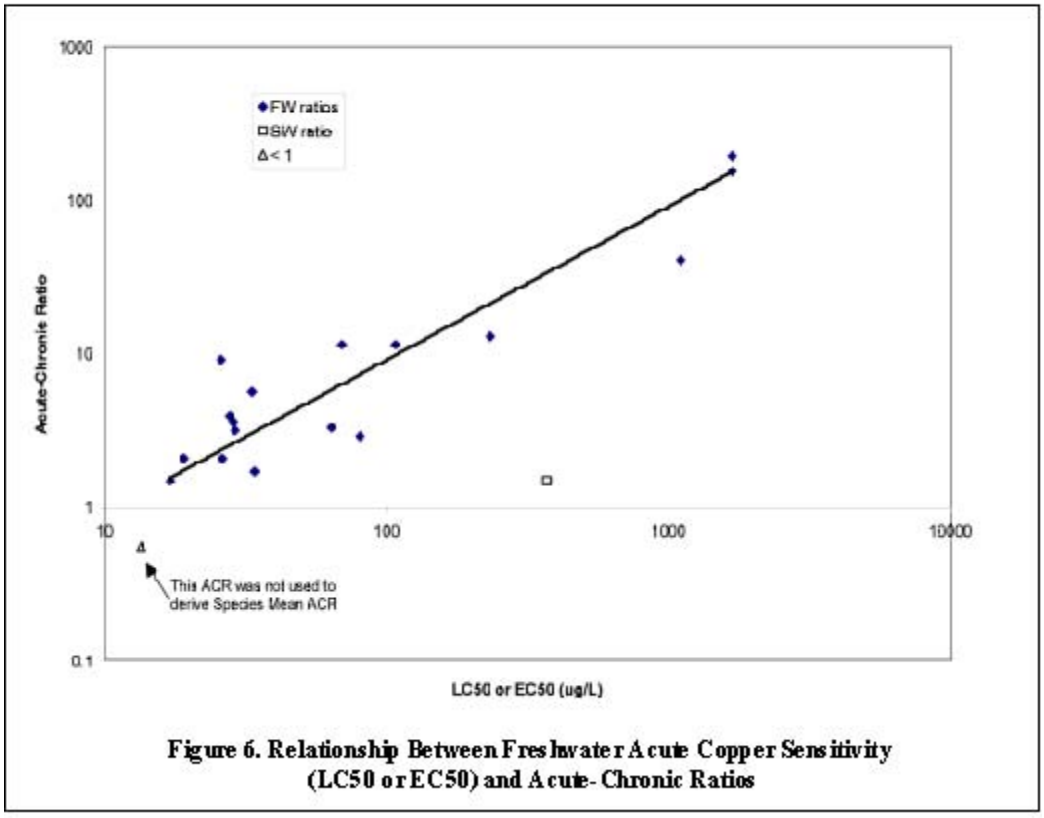

Figure 6. Relationship Between Freshwater Acute Copper Sensitivity (LC50 or EC50) and Acute-Chronic Ratios

5.0 PLANT DATA

Copper has been widely used as an algicide and herbicide for nuisance aquatic plants (McKnight et al., 1983). Although copper is known as an inhibitor of photosynthesis and plant growth, toxicity data on individual species suitable for deriving aquatic life criteria (Table 4) are not numerous.

The relationship of copper toxicity to the complexing capacity of the water or the culture medium is now widely recognized (Gächter et al., 1973; Petersen, 1982), and several studies have used algae to "assay" the copper complexing capacity of both fresh and salt waters (Allen et al., 1983; Lumsden and Florence, 1983; Rueter, 1983). It has also been shown that algae are capable of excreting complexing substances in response to copper stress (McKnight and Morel, 1979; Swallow et al., 1978; van den Berg et al., 1979). Foster (1982) and Stokes and Hutchinson (1976) have identified resistant strains and/or species of algae from copper (or other metal) impacted environments. A portion of this resistance probably results from induction of the chelate-excretion mechanism. Chelate excretion by algae may also serve as a protective mechanism for other aquatic organisms in eutrophic waters; that is, where algae are capable of maintaining free copper activities below harmful concentrations.

Copper concentrations from 1 to 8,000 µg/L have been shown to inhibit growth of various freshwater plant species. Very few of these tests, though, were accompanied by analysis of actual copper exposure concentrations. Notable exceptions are freshwater tests with green alga including *Chlamydomonas reinhardtii* (Schafer et al., 1993; Winner and Owen, 1991b), which is the only flow-through, measured test with an aquatic plant, *Chlorella vulgaris* and *Selenastrum capricornutum* (Blaylock et al., 1985). There is also a measured test with duckweed, *Lemna minor* (Taraldsen and Norberg-King, 1990).

A direct comparison between the freshwater plant data and the BLM derived criteria is difficult to make without a better understanding of the composition of the algal media used for different studies (e.g., DOC, hardness, and pH) because these factors influence the applicable criteria comparison. BLM derived criteria for certain water conditions, such as low to mid-range pH, hardness up to 100 mg/L as $CaCO_3$, and low DOC are in the range of, if not lower than, the lowest reported toxic endpoints for freshwater algal species and would therefore appear protective of plant species. In other water quality conditions BLM-derived criteria may be significantly higher (see Figure 5).

Two publications provide data for the red algae *Champia parvula* that indicate that reproduction of this species is especially sensitive to copper. The methods manual (U.S. EPA 1988) for whole effluent toxicity (WET) testing contains the results of six experiments showing nominal reproduction LOECs from 48-hr exposures to 1.0 to 2.5 µg/L copper (mean 2.0 µg/L); these tests used a mixture of 50 percent sterile seawater and 50 percent GP2 medium copper. The second study by Morrison et al. (1989) evaluated interlaboratory variation of the 48-hr WET test procedure; this six-test study gave growth EC50 values from 0.8 to 1.9 µg/L (mean 1.0 µg/L). Thus, there are actually 12 tests that provide evidence of significant reproductive impairment in *C. parvula* at nominal copper concentrations between 0.8 and 2.5 µg/L. For these studies though, the dilution water source was not identified.

One difficulty in assessing these data is the uncertainty of the copper concentration in the test solutions, primarily with respect to any background copper that might be found in the dilution water, especially with solutions compounded from sea salts or reagents. Thus, with a CCC of 1.9 µg/L dissolved copper, the significance of a 1 or 2 µg/L background copper level to a 1 to 3 µg/L nominal effect level can be considerable.

The reproduction of other macroalgae appears to be generally sensitive to copper, but not to the extent of *Champia*. Many of these other macroalgae appear to have greater ecological significance than *Champia*, several forming significant intertidal and subtidal habitats for other saltwater organisms, as well as being a major food source for grazers. Reproductive and growth effects on the other species of macroalgae sometimes appear to occur at copper concentrations between 5 and 10 µg/L (Appendix B, Other Data). Thus, most major macrophyte groups seem to be adequately protected by the CMC and CCC, but appear similar in sensitivity to some of the more sensitive groups of saltwater animals.

6.0 OTHER DATA

Many of the data identified for this effort are listed in Appendix B, Other Data, for various reasons, including exposure durations other than 96 hours with the same species reported in Table 1, and some exposures lasting up to 30 days. Acute values for test durations less than 96 hours are available for several species not shown in Table 1. Still, these species have approximately the same sensitivities to copper as species in the same families listed in Table 1. Reported LC50s at 200 hours for chinook salmon and rainbow trout (Chapman, 1978) differ only slightly from 96-hour LC50s reported for these same species in the same water.

A number of other acute tests in Appendix B were conducted in dilution waters that were not considered appropriate for criteria development. Brungs et al. (1976) and Geckler et al. (1976) conducted tests with many species in stream water that contained a large amount of effluent from a sewage treatment plant. Wallen et al. (1957) tested mosquitofish in a turbid pond water. Until chemical measurements that correlate well with the toxicity of copper in a wide variety of waters are identified and widely used, results of tests in unusual dilution waters, such as those in Appendix B, will not be very useful for deriving water quality criteria.

Appendix B also includes tests based on physiological effects, such as changes in appetite, blood parameters, stamina, etc. These were included in Appendix B because they could not be directly interpreted for derivation of criteria. For the reasons stated in this section above, data in Appendix B was not used for criteria derivation.

A direct comparison of a particular test result to a BLM-derived criterion is not always straightforward, particularly if complete chemical characterization of the test water is not available. Such is the case for a number of studies included in Appendix B. While there are some test results with effect concentrations below the example criteria concentrations presented in this document, these same effect concentrations could be above criteria derived for other normalization chemistries, raising the question as to what is the appropriate comparison to make. For example, Appendix B includes an EC50 for *D. Pulex* of 3.6 µg/L (Koivisto et al., 1992) at an approximate hardness of 25 mg/L (33 mg/L as $CaCO_3$). Yet, example criteria at a hardness of 25 mg/L (as $CaCO_3$) (including those in Figure 6) range from 0.23 µg/L (DOC = 0.1 mg/L) to 4.09 µg/L (DOC = 2.3 mg/L) based

on the DOC concentration selected for the synthetic water recipe. The chemical composition for the Koivisto et al. (1992) study would dictate what the appropriate BLM criteria comparison should be.

Based on the expectation that many of the test results presented in Appendix B were conducted in laboratory dilution water with low levels of DOC, the appropriate comparison would be to the criteria derived from low DOC waters. Comparing many of the values in Appendix B to the example criteria presented in this document, it appears that a large proportion of Appendix B values are above these concentration levels. This is a broad generalization though and as stated previously, all important water chemistry variables that affect toxicity of copper to aquatic organisms should be considered before making these types of comparisons.

Studies not considered suitable for criteria development were placed in Appendix G, Unused Data.

7.0 NATIONAL CRITERIA STATEMENT

The available toxicity data, when evaluated using the procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that freshwater aquatic life should be protected if the 24-hour average and four-day average concentrations do not respectively exceed the acute and chronic criteria concentrations calculated by the Biotic Ligand Model.

A return interval of 3 years between exceedances of the criterion continues to be EPA's general recommendation. However, the resilience of ecosystems and their ability to recover differ greatly. Therefore, scientific derivation of alternative frequencies for exceeding criteria may be appropriate.

8.0 IMPLEMENTATION

The use of water quality criteria in designing waste treatment facilities and appropriate effluent limits involves the use of an appropriate wasteload allocation model. Although dynamic models are preferred for application of these criteria, limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. EPA recommends the interim use of 1B3 or 1Q10 for criterion maximum concentration stream design flow and 4B3 or 7Q10 for the criterion continuous concentration design flow in steady-state models. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1991).

With regard to BLM-derived freshwater criteria, to develop a site-specific criterion for a stream reach, one is faced with determining what single criterion is appropriate even though a BLM criterion calculated for the event corresponding to the input water chemistry conditions will be time-variable. This is not a new problem unique to the BLM—hardness-dependent metals criteria are also time-variable values. Although the variability of hardness over time can be characterized, EPA has not provided guidance on how to calculate site-specific criteria considering this variability. Multiple input parameters for the BLM could complicate the calculation of site-specific criteria because of their combined effects on variability. Another problem arise from potential scarcity of data from small stream reaches with small dischargers. The EPA is currently exploring two

approaches to fill data gaps in such situations. One potential approach is the selection of values based on geography, the second approach is based on correlations between measured parameters and missing parameter measurements. A companion document in the form of Supplementary Training Materials, addressing issues related to data requirements, implementation, permitting, and monitoring will be released via EPA's website following the publication of this criteria document.□□

9.0 REFERENCES

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Appendices

Appendix A. Ranges in Calibration and Application Data Sets

Appendix B. Other Data on Effects of Copper on Freshwater Organisms

Appendix C. Estimation of Water Chemistry Parameters for Acute Copper Toxicity Tests

Appendix D. Saltwater Conversion Factors for Dissolved Values

Appendix E. BLM Input Data and Notes

Appendix F. Regression Plots

Appendix G. Example Water Quality Criteria Values Using the BLM and the Hardness Equation

Appendix H. Unused Data